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Published in:
Epilepsia

Published: 01/01/2024

Document Version:
Final Published version, also known as Publisher’s PDF, Publisher’s Final version or Version of Record

License:
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Publication record in CityU Scholars:
Go to record

Published version (DOI):
10.1111/epi.17816

Publication details:

Citing this paper
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Visually or auditorily induced seizures involve the activation of nonhippocampal brain areas and hippocampal removal does not alleviate seizures in a mouse model of temporal lobe epilepsy

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Abstract

Objective: Several studies have attributed epileptic activities in temporal lobe epilepsy (TLE) to the hippocampus; however, the participation of nonhippocampal neuronal networks in the development of TLE is often neglected. Here, we sought to understand how these nonhippocampal networks are involved in the pathology that is associated with TLE disease.

Methods: A kainic acid (KA) model of temporal lobe epilepsy was induced by injecting KA into dorsal hippocampus of C57BL/6J mice. Network activation after spontaneous seizure was assessed using c-Fos expression. Protocols to induce seizure using visual or auditory stimulation were developed, and seizure onset zone (SOZ) and frequency of epileptic spikes were evaluated using electrophysiology. The hippocampus was removed to assess seizure recurrence in the absence of hippocampus.

Results: Our results showed that cortical and hippocampal epileptic networks are activated during spontaneous seizures. Perturbation of these networks using visual or auditory stimulation readily precipitates seizures in TLE mice; the frequency of the light-induced or noise-induced seizures depends on the induction modality adopted during the induction period. Localization of SOZ revealed the existence of cortical and hippocampal SOZ in light-induced and noise-induced seizures, and the development of local and remote epileptic spikes in TLE occurs during the early stage of the disease. Importantly, we further discovered that removal of the hippocampi does not stop seizure activities in TLE mice, revealing that seizures in TLE mice can occur independent of the hippocampus.

Significance: This study has shown that the network pathology that evolves in TLE is not localized to the hippocampus; rather, remote brain areas are also recruited. The occurrence of light-induced or noise-induced seizures and epileptic
INTRODUCTION

Epilepsy is a neurological disease that is characterized by spontaneous and recurrent seizures. On a global scale, approximately 1%–2% of the human population suffers from epilepsy, and 30%–40% of these patients are resistant to drug intervention. An imbalance between neuronal excitation and inhibition at the circuit level, which distorts neuronal networks on a structural and functional scale, characterizes the epileptic brain.

Temporal lobe epilepsy (TLE) is the most common form of acquired epilepsy, often characterized by hippocampal lesions. Whether epileptic activities that characterize the lesioned hippocampus can be observed in remote brain areas in TLE and whether seizures can be initiated in these areas are yet to be clearly defined. Electrophysiological studies conducted by Sheybani et al. revealed the development of a large-scale epileptic network in a mouse model of TLE, showing the possibility of extensive macrocircuit dynamics in TLE involving nonhippocampal brain networks.

Hyperexcitability and hypersynchronous firing of a population of hippocampal neurons has been suggested to trigger epileptic seizures in TLE. However, relapse of seizure after the surgical removal of the epileptic hippocampus in drug-resistant TLE patients reveals the possible existence of secondary seizure foci outside the hippocampus and questions the hippocampus as the sole initiator of epileptic seizure in TLE. The possibility of TLE being a macrocircuit disease cannot be overlooked. Hence, there is a need to understand the participation of nonhippocampal networks in TLE.

Visual and auditory auras are often reported in some TLE patients. Studies have shown that such auras usually point to the onset of a tonic–clonic seizure, thereby suggesting the participation of cortical networks in TLE pathology. Clinical evaluation of some epileptic patients has also shown that several precipitating factors can contribute to the initiation of seizures in epileptic individuals, and some epileptic patients have seizures that can be initiated by light or noise stimulation. To elucidate the role of nonhippocampal networks, particularly the cortex, in TLE on a behavioral and physiological scale, there is a need to develop TLE animal models with seizures that can be induced by exposure to external stimulus. Whether TLE mice can show seizures in response to light or noise stimuli, which can reveal the involvement of cortical networks in TLE, is yet to be determined.

This study, therefore, aims to understand the active participation of nonhippocampal networks, particularly the cortex, in TLE pathology and how such activity predisposes cortical networks to generate epileptic seizures.

2 MATERIALS AND METHODS

2.1 Animals

Adult male C57BL/6J aged 7–8 weeks and weighing 20–25 g obtained from the City University of Hong Kong Laboratory Animal Research Holding Unit maintained at a 12-h light/12-h dark cycle (dark from 08:00 to 20:00) with food and water provided ad libitum were used for experiments. Mice that were video recorded 24/7 were housed individually in cages, whereas those that were not subjected to continuous video recording were housed...
in a group of five mice per cage in accordance with the university’s animal holding unit policy under an optimal housing condition. All experimental procedures were approved by the Animal Subjects Ethics Subcommittee of the City University of Hong Kong. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guideline was adhered to in the design, data collection, analysis, and reporting of results.

For surgical procedures, mice were anesthetized with pentobarbital (50 mg/kg ip, Ceva Sante Animale Co.). Anesthetized mice were placed on stereotaxic equipment (RWD, Life Sciences), followed by an incision on the scalp. Bregma and lambda positions were balanced, and appropriate craniotomies were made based on the target brain region.

## 2.2 Kainic acid injection

Mice underwent the surgical procedure outlined above. After craniotomy, 650 nL of .3 mmol·L\(^{-1}\) kainic acid (KA; Cat. No. 0222; Tocris Bioscience) was injected at a speed of 50 nL/min (Nanoliter Injector, World Precision Instruments) into the right dorsal hippocampus (coordinates: anteroposterior [AP] = −2.06 mm, mediolateral [ML] = −1.80 mm, and dorsoventral [DV] = −1.60 mm) of mice to induce TLE. The injection needle was kept in place for an additional 5 min to allow for local diffusion of KA before its withdrawal. Mice developed status epilepticus (SE) after KA injection, and the onset of SE after KA injection was marked by spinning around, facial automatisms, forelimb clonus, loss of postural balance, and frequent convulsions of mice (Movie S3) as previously described.\(^{20}\) SE was allowed to self-terminate. We did not perform additional acclimatization of mice before epilepsy induction. Hence the variations in seizure frequency recorded among mice could be a consequence of lack of acclimatization.

## 2.3 c-Fos expression

Mice were video recorded 24/7 after hippocampal KA injection. To identify the moment of spontaneous seizure for c-Fos expression immunohistochemistry, epileptic mice were video monitored for an additional 3 weeks after their first spontaneous seizure. Mice were perfused 50 min after having spontaneous seizure for c-Fos expression, and epileptic mice without spontaneous seizure in the past 24 h were perfused as control. Details about c-Fos immunohistochemistry can be found in Data S1.

## 2.4 Light, noise, or no induction protocol

For light induction after KA injection, 18 KA-injected mice designated TLE+Light were exposed daily for a period of 14 days post-KA injection to a 20-Hz blue flashlight with a stimulation period of 600 ms and 10-s intertrial interval for 10 h per day. The flashlight was generated by a direct current-driven torch bulb from the voltage output of an RZ5 Bioamp Processor (Tucker and Davies Technology [TDT]). Light intensity corresponding to a trigger voltage of 3.80 V was used.

For noise induction after KA injection, a new cohort of 11 KA-injected mice designated TLE+Noise were exposed daily for a period of 14 days to 65-dB white noise (600-ms noise duration with an intertrial interval of 10 s) at 10 h per day. The noise was delivered via a free-field magnetic speaker (MF-1, TDT) generated by the RZ6 Multi I/O processor (TDT).

However, for no induction, 16 mice newly injected with KA designated TLE were kept in the experimental chamber for 10 h per day for a duration of 14 days without exposing them to any stimulus induction, that is, no light or noise stimulation.

These induction or no induction protocols were conducted during the dark cycle phase for mice (dark from 08:00 to 20:00).

## 2.5 Light and noise test for behavioral seizure

The mice were tested for light-induced and noise-induced seizures after the light, noise, or no induction period was over. Each test session (a total of 20 test sessions) was divided into three epochs consisting of before, during, and after stimulation. For each test session, the mouse was placed in an acrylic test box in a light- and sound-proof chamber for 25 min without any stimulation, after which 50 min of 20-Hz flashlight stimulation of 600-ms duration with an intertrial interval of 10 s\(^{21}\) or 65-dB noise stimulation (600-ms noise duration with an intertrial interval of 10 s) for the noise experiment was presented to the animal, followed by 50 min of no stimulation. The behavior of mice during the test session was recorded using two cameras: a night-vision and a non-night vision camera. Test sessions were conducted during the dark cycle phase of mice (08:00–20:00). The variations in the time of the day that the seizures were triggered could have influenced the extent of seizure induction as well as the variation in seizure frequency recorded in our study.
Convulsive behavioral seizures corresponding to Racine scales 4 and 5 were recorded. Behavioral seizures that occurred during or after light stimulation were termed light-induced seizures, and those that occurred during or after noise stimulation were termed noise-induced seizures.

### 2.6 **Microelectrode and cannula implantation**

Nine of the 18 mice subjected to the light induction protocol (TLE+Light-Ephys mice 10–18) and eight of the 16 mice with no induction (TLE-Ephys mice 9–16) were also implanted with custom-made chronic tungsten microelectrodes (tungsten wire, California Fine Wire) immediately after KA injection. After intrahippocampal KA injection and removal of dura using a 29-gauge syringe needle bent into a hook-shape, individual microelectrodes were implanted at the following stereotaxic coordinates relative to bregma: motor cortex (MCx; AP: +1.50 mm, ML: −1.80 mm, DV: −.70 mm), hippocampus (AP: −2.15 mm, ML: −1.80 mm, DV: −1.40 mm), visual cortex (VCx; AP: −2.50 mm, ML: −4.10 mm, DV: −.70 mm), and auditory cortex (ACx; AP: −2.50 mm, ML: −4.10 mm, DV: −.60 mm). Each microelectrode was carefully lowered into these individual brain regions using the micromanipulator. Screw electrodes (1.0 × 2.0 mm) were used for a common reference placed at the ipsilateral frontal bone, ground electrode was placed at the contralateral frontal bone, and individual silver cable connected to the electrode pins was wound around these screw electrodes. Additional screws were fastened each on opposite sides of the cranial bone above the cerebellum to balance the frontal screws. Kwik-Cast (silicone sealant, World Precision Instruments) was used to cover the exposed brain tissue around the electrodes after implantation, and dental cement mixed with liquid base (mega PRESS NV + JET X, megadental) was used to cover the skull.

For the experiment involving monitoring local field potential (LFP) activities from the baseline to the early post-KA and late post-KA periods, one of the electrodes to be implanted into the hippocampus was coupled to a drug-infusion guide cannula (OD.0.48 mm-26G/M3.5, Cat. No. 62003, RWD, Life Sciences) to record as closely as possible to the KA infusion site. Individual electrodes were implanted into the MCx (AP: 1.50 mm, ML: −1.80 mm, DV: −.70 mm) and VCx (AP: −4.00 mm, ML: −2.50 mm, DV: −.60 mm), and the guide cannula coupled with an electrode was implanted into the hippocampus (AP: −2.06 mm, ML: −1.80 mm, DV: −1.50 mm). Mice were allowed to recover from surgery for at least 1 week before neuronal activities were recorded.

### 2.7 **Surgical removal of hippocampus**

The KA-injected hippocampus was surgically removed after baseline spontaneous seizure was recorded for 7 days. For removal of the KA-injected epileptic hippocampus, stereotaxic coordinates were marked on the skull (AP: −1.30 mm, ML: −1.80 mm; AP: −2.70 mm, ML: −1.80 mm; AP: −2.0 mm, ML: −1.0 mm; AP: −2.0 mm, ML: −3.0 mm) and a circle was drawn to link the coordinates. Craniotomy was performed, followed by aspiration from cortex to hippocampus. The space left by the aspirated tissue was filled with Kwik-Cast (World Precision Instruments). Animals were returned to individual cages for video recording after recovery from surgery and were administered with carprofen and dexamethasone. Mice were monitored with continuous video recording for 3 weeks after hippocampus removal. Epileptic mice without removal of KA-injected hippocampus served as control and was also video monitored.

### 2.8 **Ibotenic acid injection**

Ibotenic acid (IBO; 10 mg/mL; ab120041, Abcam) was injected into the bilateral hippocampus (coordinates AP: −1.80 mm, ML: ± 1.30 mm, DV: −1.60 mm; AP: −2.70 mm, ML: ± 2.80 mm, DV: −1.85 mm; AP: −3.50 mm, ML: ± 3.0 mm, DV: −2.80 mm) of epileptic mice previously injected with KA at a volume of 200 nL per site with a speed of 50 nL/min after obtaining 7 days baseline video recording of spontaneous seizure. The mice were returned to the cages and were housed individually for continuous video monitoring. Mice were continuously video monitored for 3 weeks after IBO injection. Epileptic mice without IBO injection served as control and were also video monitored.

### 2.9 **Histology, immunohistochemistry, and imaging**

At the end of the experiments, mice were overdosed with pentobarbital and perfused with 1 × PBS, followed by fixation with 4% paraformaldehyde. After serial dehydration of the brains in 10%, 20%, and 30% PBS sucrose, coronal sections with a thickness of 50 μm were taken using a cryostat (Leica CM3050 S, Leica Biosystems). Comprehensive details can be found in Data S1.
2.10  Data analysis

Custom algorithms written in MATLAB (R2018b, MathWorks) were used to analyze electrophysiological data. Figures were plotted using MATLAB and Origin 2018 (OriginLab). Comprehensive details on data analysis can be found in Data S1.

2.11  Statistics

Grouped data are expressed as mean ± SD. Statistical comparisons, namely, one-way and two-way repeated-measures (RM) analysis of variance (ANOVA) with Bonferroni multiple comparisons test, were performed with GraphPad Prism (version 8.0.2). Statistical significance level was set at \( p < .05 \), \( p < .01 \), and \( p < .001 \).

3  RESULTS

3.1  Cortical and hippocampal neurons are recruited during spontaneous seizures in TLE

To elucidate whether network activation during spontaneous seizures occurs within the hippocampus only or whether nonhippocampal brain areas are also recruited in TLE mice, we adopted the KA model as a mouse model of TLE. Mice were flocally injected with KA in the right dorsal hippocampus to establish TLE followed by continuous video monitoring. TLE mice were perfused 50 min after having a spontaneous seizure to ascertain the c-Fos expression pattern, and TLE mice without seizures in the past 24 h were perfused as control (Figure 1A). The average latent period for mice perfused after having a spontaneous seizure was \( 10 ± 1.73 \text{ days} \) (range \( 8–11 \text{ days} \)), and that of TLE mice perfused without having a spontaneous seizure in the past 24 h was \( 10.7 ± 2.73 \text{ days} \) (range \( 6–19 \text{ days} \)). Histology revealed apparent structural alterations in the KA-injected hippocampus (Figure 1B). Staining for the immediate early gene c-Fos in TLE mice without seizures reveals a baseline c-Fos expression in the hippocampus and nonhippocampal structures of TLE mice (Figure 1C, left; insets i–iv are magnified in Figure 1D, top). On the other hand, an intense and prominent labeling of Fos+ cells characterized the hippocampal and nonhippocampal structures after spontaneous seizure (Figure 1C, right, insets i–iv are magnified in Figure 1D, bottom), which signifies local and remote neuronal network hyperexcitation caused by seizure. Nonhippocampal structures of interest are MCx, VCx, and ACx. Group quantification of the number of c-Fos+ cells reveals enhanced c-Fos expression in TLE mice that had spontaneous seizures compared with the group without seizures, and this increase was consistent in the structures of interest (Figure 1E; two-way ANOVA, \( F_{3,32} = 1.031, p = .3920 \), Bonferroni multiple comparisons; MCx, no spontaneous seizure in the past 24 h vs. after spontaneous seizure \( = 198.85 ± 75.87 \text{ vs. } 435.13 ± 95.39 \text{ c-Fos}^+ \text{ cells/mm}^2, p < .001 \); VCx, no spontaneous seizure in the past 24 h vs. after spontaneous seizure \( = 239.45 ± 53.77 \text{ vs. } 526.26 ± 122.18 \text{ c-Fos}^+ \text{ cells/mm}^2, p < .001 \); and hippocampus, no spontaneous seizure in the past 24 h vs. after spontaneous seizure \( = 348.42 ± 49.93 \text{ vs. } 659.61 ± 108.11 \text{ c-Fos}^+ \text{ cells/mm}^2, p < .001 \); and hippocampus, no spontaneous seizure in the past 24 h vs. after spontaneous seizure \( = 178.89 ± 51.56 \text{ vs. } 455.48 ± 82.77 \text{ c-Fos}^+ \text{ cells/mm}^2, p < .001 \). This result implies that seizure activity in TLE efficiently propagates from the seizure onset zone (SOZ) to other brain regions. Cortical and hippocampal neuronal activation during spontaneous seizure reveals the existence of a corticohippocampal epileptic network in TLE mice.

3.2  Light and noise stimulation triggers epileptic seizures in epileptic mice

Visual and auditory auras are often reported in some TLE patients. These auras usually serve as a warning sign for an upcoming tonic–clonic seizure, suggesting that TLE disorder also affects the cortex. Having found the expression of c-Fos in sensory cortices, especially in the visual and auditory cortices after a spontaneous seizure, we therefore reasoned that a hyperexcitable epileptic network is formed in the cortex of TLE mice, and that the activation of this network using sensory stimuli such as light or noise could trigger seizure in epileptic mice. To test our hypothesis, we subjected mice to a light induction, noise induction, or no induction protocol for days 1–14 after hippocampal KA injection, after which we examined these mice for light-induced and noise-induced seizures (Figure 2A). TLE mice subjected to light induction were designated TLE+Light mice, those subjected to noise induction protocol were designated TLE+Noise mice, and those that were not subjected to any induction protocol were designated TLE mice.

3.2.1  TLE plus light induction

We found that TLE+Light mice developed convulsive seizures during and after light stimulation, with a very sparse occurrence of seizures before light stimulation (Figure 2B, left; total number of seizures before stimulation \( = 3 \), during stimulation \( = 36 \), after stimulation \( = 37 \), \( n = 18 \) mice;
(A) Intrahippocampal KA Injection

\[ \text{Latent Phase} \rightarrow \text{~2-3 weeks} \rightarrow \text{3 weeks} \]

**First Spontaneous Seizure**

**No spontaneous seizure in the past 24hrs**

Perfuse for c-Fos expression

50 minutes

(B) Nissl

(C) DAPI c-FOS

No spontaneous seizure in the past 24hrs

After spontaneous seizure

(E) c-Fos+ cells/mm²

No spontaneous seizure in the past 24hrs

After spontaneous Seizure

Motor Cortex

Visual Cortex

Auditory Cortex

Hippocampus

(D) i: motor cortex

ii: hippocampus

iii: auditory cortex

iv: visual cortex

No spontaneous seizure in the past 24hrs

After spontaneous seizure

**c-FOS**
3.2.2 | TLE plus noise induction

To ascertain whether the induction modality used in days 1–14 after KA injection influences the frequency of light-induced and noise-induced seizures, we switched the induction modality during the induction period to noise induction in a new cohort of KA-injected mice designated TLE+Noise. As expected, TLE+Noise mice developed noise-induced seizures in response to noise stimulation (Figure 2C, center; total number of seizures before stimulation = 1, during stimulation = 19, after stimulation = 15, n = 11 mice; one-way RM ANOVA, F2, 20 = 13.94, p = .0002; Bonferroni multiple comparisons, before noise: .011 ± .036 vs. during noise: .104 ± .093, p = .0002; after noise: .011 ± .036 vs. after noise: .082 ± .082, p = .0029; during noise: .104 ± .093 vs. after noise: .082 ± .082, p = .7460; seizure frequency is expressed as seizures/h), similarly, light stimulation also evoked light-induced seizures in these mice (Figure 2C, left; total number of seizures before stimulation = 2, during stimulation = 10, after stimulation = 6, n = 11 mice; one-way RM ANOVA, F2, 20 = 1.547, p = .2373; Bonferroni multiple comparisons, before light: .022 ± .049 vs. during light: .055 ± .063, p = .2986; after light: .022 ± .049 vs. after light: .033 ± .041, p = .9999; during light: .055 ± .063 vs. after light: .033 ± .041, p = .7893; seizure frequency is expressed as seizures/h). Comparison of seizure frequency before light and before noise stimulation showed no statistical difference; in contrast to the light induction results, we found a significant increase in the frequency of noise-induced seizures during noise stimulation compared with during light stimulation and also a significant increase in seizure frequency of noise-induced seizures was recorded after noise stimulation compared with after light stimulation (Figure 2C, right; two-way RM ANOVA, F2, 30 = 3.805, p = .0337; Bonferroni multiple comparisons, before light: .022 ± .049 vs. before noise: .011 ± .036, p = .9999; during light: .055 ± .063 vs. during noise: .104 ± .093, p = .0290; after light: .033 ± .041 vs. after noise: .082 ± .082, p = .0290; n = 11 mice; seizure frequency is expressed as seizures/h).

3.2.3 | TLE, no stimulus induction

To confirm whether our two previous induction protocols contributed to the increase in the susceptibility of TLE+Light and TLE+Noise mice to have increased occurrence of light-induced or noise-induced seizures, we
further developed a third group of mice that were not subjected to any stimulus induction in days 1–14 after KA injection, designated TLE mice. The light and noise test sessions were conducted in these mice after 14 days. The results show that there was no statistically significant change in seizure frequency during and after light compared with before light stimulation in the light test session for the TLE mice (Figure 2D, left; total number of seizures before stimulation = 1, during stimulation = 8, after stimulation = 6, n = 16 mice; one-way RM ANOVA, $F_{2, 30} = .9633$, $p = .931$; Bonferroni multiple comparisons, before light: $0.075 \pm .03$ vs. during light: $0.03 \pm .05$, $p = .5491$; before light: $0.075 \pm .03$ vs. after light: $0.023 \pm .08$, $p < .00001$; during light: $0.03 \pm .05$ vs. after light: $0.023 \pm .08$, $p < .00001$; seizure frequency is expressed as seizures/h); similarly, no statistically significant change in seizure frequency was recorded during and after noise compared with before noise stimulation in these TLE mice (Figure 2D, center; total number of seizures before stimulation = 0, during stimulation = 4, after stimulation = 7, one-way RM ANOVA, $F_{2, 30} = 2.079$, $p = .1427$; Bonferroni multiple comparisons, before noise: $0 \pm 0$ vs. during noise: $0.015 \pm .05$, $p = .7642$; before noise: $0 \pm 0$ vs. after noise: $0.026 \pm .04$, $p = .1533$; during noise: $0.015 \pm .05$ vs. after noise: $0.026 \pm .04$, $p < .0005$; n = 16 mice; seizure frequency is expressed as seizures/h). Contrary to the TLE+Light and TLE+Noise mice, group analysis showed there is no statistically significant difference between the frequency of seizures that occurred in the light test session and seizures that occurred in the noise test session in the TLE mice (Figure 2D, right; two-way RM ANOVA, $F_{2, 30} = .1298$, $p = .8788$; Bonferroni multiple comparisons, before light: $0.075 \pm .03$ vs. before noise: $0 \pm 0$, $p > .9999$; during light: $0.03 \pm .05$ vs. during noise: $0.015 \pm .05$, $p > .9999$; after light: $0.023 \pm .08$ vs. after noise: $0.026 \pm .04$, $p > .9999$; $n = 16$ mice; seizure frequency is expressed as seizures/h). The absence of a significant increase in seizure frequency after exposing TLE mice to light and noise test sessions reveals that sensory stimulation is insufficient in inducing seizures in TLE mice that were not exposed to sensory induction during the induction period. This signifies that sensory induction during the induction period is essential for TLE+Light and TLE+Noise mice to have increased vulnerability for light-induced and noise-induced seizures, and the absence of such sensory induction makes TLE mice unable to have sensory-induced seizures.

Furthermore, comparison of seizure frequency among the TLE, TLE+Light, and TLE+Noise groups in the light test session further validates the critical influence of the light induction protocol in making TLE+Light mice have more light-induced seizures compared with the TLE+Noise and TLE groups (Figure 2E, left; two-way RM ANOVA, $F_{4, 84} = 2.930$, $p = .0255$; Bonferroni multiple comparisons, before light: TLE vs. TLE+Light = $0.0075 \pm .03$ vs. $0.02 \pm .06$, $p > .9999$, TLE vs. TLE+Noise = $0.0075 \pm .03$ vs. $0.0249$, $p < .0001$, TLE+Light vs. TLE+Noise = $0.02 \pm .049$ vs. $p < .9999$; after light: TLE vs. TLE+Light = $0.0249 \pm .049$ vs. $p > .9999$, TLE vs. TLE+Noise = $0.02 \pm .049$ vs. $p < .9999$, TLE+Light vs. TLE+Noise = $0.02 \pm .049$ vs. $p > .9999$; $n = 16$ mice). Data are expressed as mean ± SD. (C) Distribution of light-induced and noise-induced seizure frequency increase in epileptic mice after light or noise induction period. (A) Experimental protocol for stimulus-induced seizures consisting of three stages: (i) kainic acid (KA) injection; (ii) light, noise, or no induction; and (iii) test sessions. Mice were subjected to 14 days of light induction (TLE+Light)/noise induction (TLE+Noise)/no stimulus induction (temporal lobe epilepsy [TLE]) for 10 h per day after KA injection. Test sessions began at the end of day 14, and in the test session, mice were exposed to either light stimulation for the light stimulation test session or noise for the noise stimulation test session. Each test session consisted of 25 min of no stimulation then 50 min of light or noise stimulation followed by 50 min of no stimulation. (B) TLE+plus light induction. Distribution of light-induced seizure (left), noise-induced seizure (center) and group average of seizures (right) before, during, and after light or noise stimulation in TLE+Light. Each shape represents an individual mouse. Grouped data show the significant increase in the frequency of light-induced seizures compared with noise-induced seizures after 14 days of light induction (n = 18 mice, $p < .05$, ***p < .001; NS, nonsignificant). Data are expressed as mean ± SD. (C) TLE+ plus noise induction. Distribution of light-induced seizures (left), noise-induced seizures (center), and group average of seizures (right) before, during, and after light or noise stimulation in TLE+Noise mice. Each shape represents an individual mouse. Grouped data reveal the significant increase in the frequency of noise-induced seizures compared with light-induced seizures after 14 days of noise induction (n = 11 mice, $p < .05$). Data are expressed as mean ± SD. (D) TLE, no stimulus induction. Distribution is shown of light-induced seizures (left), noise-induced seizures (center), and group average of seizures (right) before, during and after light or noise stimulation in TLE mice. Each shape represents an individual mouse. Grouped data reveal no significant difference in the frequency of light-induced seizures compared with noise-induced seizures after 14 days without any stimulus induction (n = 16 mice). Data are expressed as mean ± SD. (E) Comparison of seizure frequency among the TLE+Light, and TLE+Noise mice in the light test session (left) and in the noise test session (right). Grouped data show a significant influence of the light induction protocol in making TLE+Light mice have more light-induced seizures compared with the TLE and TLE+Noise groups. Also, grouped data revealed a significant effect of noise induction protocol in making TLE+Noise mice have more noise-induced seizures when compared with the TLE and TLE+Light groups (no induction, n = 16; light induction, n = 18; noise induction, n = 11; seizure frequency is expressed as seizures/h; $p < .05$, **p < .01 ***p < .001). Data are expressed as mean ± SD.
Sensory stimuli could not sufficiently induce seizures in TLE mice. On the other hand, sensory stimulus subsequently. On the other hand, sensory stimulus could not sufficiently induce seizures in TLE mice to a specific sensory stimulus after KA injection or light induction groups (Figure 2E, right; two-way RM ANOVA, $F_{4,84} = 3.197$, $p = .0170$; Bonferroni multiple comparisons, before noise: TLE vs. TLE+Light $= 0 \pm 0$ vs. $0 \pm 0$, $p > .9999$, TLE vs. TLE+Noise $= 0 \pm 0$ vs. $0 \pm 0.036$, $p > .9999$, TLE+Light vs. TLE+Noise $= 0 \pm 0$ vs. $0 \pm 0.036$, $p > .9999$; during noise: TLE vs. TLE+Light $= 0.015 \pm 0.03$ vs. $0.01 \pm 0.036$, $p > .9999$, TLE vs. TLE+Noise $= 0.15 \pm 0.05$ vs. $0.14 \pm 0.039$, $p = .003$, TLE+Light vs. TLE+Noise $= 0.03 \pm 0.066$ vs. $0.14 \pm 0.093$, $p = .0024$; after noise: TLE vs. TLE+Light $= 0.026 \pm 0.04$ vs. $0.06 \pm 0.077$, $p = .2457$, TLE vs. TLE+Noise $= 0.026 \pm 0.082 \pm 0.082$, $p = .0376$, TLE+Light vs. TLE+Noise $= 0.06 \pm 0.077$ vs. $0.08 \pm 0.082$, $p = .9321$, TLE, $n = 16$; TLE+Light, $n = 18$; TLE+Noise, $n = 11$; seizure frequency is expressed as seizures/h).

These results demonstrate that seizures can be initiated in TLE+Light and TLE+Noise mice when they are exposed to sensory stimuli and that the exposure of these mice to a specific sensory stimulus after KA injection increases their susceptibility to have higher frequency of seizures that can be precipitated when exposed to that sensory stimulus subsequently. On the other hand, sensory stimuli could not sufficiently induce seizures in TLE mice, suggesting that the seizures recorded in TLE mice are spontaneous seizures.

### 3.3 Light-induced and noise-induced seizures in TLE mice have cortical and hippocampal onsets

Having established that seizures in TLE+Light mice can be precipitated using sensory stimulation (light or noise) and that seizures cannot be precipitated by sensory stimulation in TLE mice, we further sought to elucidate whether these seizures are initiated in hippocampal or nonhippocampal brain areas such as sensory cortices. To identify the SOZ of light- and noise-induced seizures in TLE+Light mice (TLE+Light-Ephys mice, see Materials and Methods) and the SOZ of seizures recorded in the TLE mice (TLE-Ephys mice, see Materials and Methods), mice were implanted with chronic microelectrodes in the MCx, VCx, ACx, and hippocampus for combined behavior and electrophysiology (Figure 3A).

Electrographic seizures recorded from these mice were analyzed, and SOZ was identified using a power-based analytical method. Seizure onset was marked by low-voltage fast onset or hypersynchronous onset that developed into high-amplitude and high-frequency discharges. LFP power analysis of the first 5 s after seizure onset was used to identify the SOZ.

**Figure 3** Cortical and hippocampal seizure onset zones (SOZs) in temporal lobe epilepsy (TLE) mice subjected to light induction and TLE mice with no induction period. (A) Experimental protocol for combined behavior and electrophysiology to ascertain SOZ. TLE+Light mice that were subjected to the light induction protocol and TLE mice that were not subjected to any stimulus were also implanted with electrodes. Electrodes were implanted in the motor cortex (MCx), visual cortex (VCx), auditory cortex (ACx), and hippocampus (HPC). Mice were tested for light-induced and noise-induced seizures using the test protocol. (B) Illustrative light-evoked and noise-evoked potentials from the recorded brain regions (average of 300 stimulus repetitions). Blue and purple vertical lines indicate light and noise onset, respectively. The VCx and ACx showed characteristic high-amplitude light-evoked and noise-evoked potentials, respectively. (C) Illustrative example of visual cortex SOZ. Panels i–v show the raw LFP seizure trace, expanded seizure onset time, power spectrum of the seizure onset time, power spectral density, and averaged power spectral density, respectively. (D) Illustrative example of motor cortex SOZ. Panels i–v show the raw LFP seizure trace, expanded seizure onset time, power spectrum of the seizure onset time, power spectral density, and averaged power spectral density, respectively. (E) Illustrative example of auditory cortex SOZ. Panels i–v show the raw LFP seizure trace, expanded seizure onset time, power spectrum of the seizure onset time, power spectral density, and averaged power spectral density, respectively. (F) Illustrative example of hippocampus SOZ. Panels i–v show the raw LFP seizure trace, expanded seizure onset time, power spectrum of the seizure onset time, power spectral density, and averaged power spectral density, respectively. (G) Illustrative example of uncertain SOZ. Panels i–v show the raw LFP seizure trace, expanded seizure onset time, power spectrum of the seizure onset time, power spectral density, and averaged power spectral density, respectively. (H) Distribution of SOZ in TLE+Light mice. Upper panel shows the distribution of SOZ, that is, the number of SOZs and the number of seizures arising from these SOZs in each of the mice recorded in light-induced seizures (left) and noise-induced seizures (right) in mice 10–18, which were subjected to the light induction protocol. Each shape represents an individual mouse. Existence of multiple SOZs is readily observable in some of the mice. Lower panel shows group percentage distribution of the SOZ of light-induced seizures (left) and noise-induced seizures (right) recorded from the MCx, VCx, ACx, and HPC and of uncertain onset from the mice in the upper panel ($n = 9$ mice). (I) Distribution of SOZs in TLE mice. Upper panel shows the distribution of SOZs and the number of seizures arising from these SOZs in each of the TLE mice 9–10 which did not undergo any stimulus induction protocol. Each shape represents an individual mouse. Existence of multiple SOZs is readily observable in some of the mice. Lower panel shows group percentage distribution of the SOZs of seizures recorded in light test session (left) and seizures recorded in noise test session (right) recorded from the MCx, VCx, ACx, and HPC and of uncertain onset ($n = 8$ mice).
3.3.1 | SOZ distribution in TLE plus light induction mice

We recorded evoked potentials in response to light or noise stimulus in the MCx, VCx, ACx, and hippocampus. The VCx and ACx showed high-amplitude evoked potential in response to light and noise stimulation, respectively (Figure 3B). By using LFP power analysis, we identified various SOZs in light-induced and noise-induced seizures from mice subjected to light induction and those with no induction. Illustrative examples of the categories of SOZ identified, that is, MCx, VCx, ACx, hippocampus, and uncertain SOZ, are indicated in Figure 3C–G, respectively. We observed an interindividual variability in the SOZ among TLE+Light mice (Figure 3H, top) and in TLE mice (Figure 3I, top), as some mice showed single or multiple SOZs. The majority of light-induced seizures recorded from TLE+Light mice were from the VCx (33%, 9/27 seizures), followed by the hippocampus (26%, 7/27 seizures), MCx (22%, 6/27 seizures), and ACx (11%, 3/27 seizures), and 7% (2/27 seizures) had an uncertain onset zone (Figure 3H, bottom left; n = 9 mice). However, for the distribution of noise-induced seizures recorded in these TLE+Light mice, 31% (5/16 seizures) had their onset zone in the ACx, followed by the MCx and VCx (25%, 4/16 seizures each) and hippocampus (13%, 2/16 seizures), and 6% (1/16 seizures) had an uncertain onset zone (Figure 3H, bottom right; n = 9 mice).

3.3.2 | SOZ distribution in TLE no stimulus induction mice

On the other hand, the seizures recorded in TLE mice in the light test session had 70% (7/10 seizures) of SOZs in the hippocampus, whereas each 10% of the seizures were initiated in MCx, initiated in VCx, and of uncertain onset (1/10 seizures each; Figure 3I, bottom left; n = 8 mice). Also, 67% (4/6 seizures) of seizures in the TLE mice in the noise test session were initiated from the hippocampus, whereas 33% (2/6 seizures) of seizures were initiated in multiple brain areas (uncertain SOZ) in these mice (Figure 3I, bottom right; n = 8 mice). In addition, the distribution of SOZs per mouse can be found in Table S1. This result reveals the dominance of the cortex as the SOZ in light-induced and noise-induced seizures in the TLE+Light mice. In contrast, the hippocampus is the dominant SOZ for seizures recorded in the TLE mice, which is consistent with the previous findings by Bragin et al. and Sheybani et al. It could therefore mean that network remodeling occurs in response to sensory induction 1–14 days post-KA injection in TLE+Light mice, and this possibly makes the cortex more hyperexcitable in response to light or noise stimulus in the later stage of epilepsy in these mice. On the other hand, because there was no significant influence of sensory stimulation on seizure frequency in TLE mice, it therefore means that the seizures observed in these mice are spontaneous seizures and are not sensory induced, hence the dominance of the hippocampus as the SOZ in these mice, which is common with spontaneous seizures in TLE mice.

3.4 | Epileptic spikes develop in the cortex and hippocampus of mice during the early period and progress to the late period after KA infusion

Based on our observation of epileptic discharges in LFP recorded in cortical areas remote from the hippocampus in TLE mice, we further asked whether these LFP epileptic discharges can be observed in the early and later days after KA infusion. To this end, we examined the evolution of epileptic spikes in the hippocampus and cortex from the baseline physiology to days 1–7 and days 25–28 post-KA infusion in mice by combining multiple-site in vivo recording with infusion cannula. After electrodes and cannula implantation, mice were allowed to recover for 7 days. Baseline brain LFP activity was recorded daily for 4 days followed by KA infusion. Further recordings were done from days 1 to 7 (early post-KA phase) and days 25–28 (late post-KA phase) after KA infusion (Figure 4A). Electrodes and cannula placement were confirmed histologically (Figure S1). Figure 4B shows illustrative LFP traces in the baseline (left), early post-KA (center), and late post-KA (right). The baselines before KA infusion were relatively stable across days in the hippocampus, VCx, and MCx (Figure 4C; days −4 to −1). Surprisingly, a striking increase in the frequency of LFP (epileptic) spikes was observed in the hippocampus, VCx, and MCx as early as day 1 after hippocampal KA infusion, revealing the onset of local and remote network pathology, and the frequency of this activity stayed above baseline in the early days after KA infusion (Figure 4C; days 1–7). The increased hippocampal and cortical spiking activity was further sustained until the later stage post-KA infusion (Figure 4C; days 25–28). Group average showed a significant increase in the frequency of epileptic spikes from baseline to the early post-KA and late post-KA periods in the hippocampus (Figure 4D, left; one-way RM ANOVA, F(2,16) = 16.41, p < .001; Bonferroni multiple comparisons, baseline vs. early post-KA = 1.286 ± .39 vs. 4.899 ± 1.88 spikes/min, p = .0272; baseline vs. late post-KA = 1.286 ± .39 vs. 8.258 ± 3.94 spikes/min, p < .001; early post-KA vs. late post-KA = 4.899 ± 1.88 vs. 8.258 ± 3.94 spikes/min, p = .0419; n = 9 mice), VCx (Figure 4D, middle; one-way
Development of cortical and hippocampal epileptic activities after hippocampal kainic acid (KA) infusion. (A) Experimental protocol to evaluate the development of epileptic spikes in the hippocampus and cortex. After electrode and cannula implantation, mice were allowed to recover for 7 days, after which they were recorded for 4 days to obtain baseline values of LFP spikes. KA was infused into the hippocampus on day 0, and LFP recording was resumed the next day. Mice were recorded for days 1–7 corresponding to the early post-KA period, after which recording was discontinued. LFP recording was resumed on day 25–28 corresponding to the late post-KA period. HPC, hippocampus; MCx, motor cortex; VCx, visual cortex. (B) Illustrative raw LFP traces of epileptic spike development in baseline (left), early post-KA (center), and late post-KA (right). Fewer spikes can be seen in the baseline signal; in contrast, increased frequency of epileptic spikes began to appear in the early phase after KA infusion and the discharge continued into the later phase post-KA infusion in all the recorded brain areas. (C) The daily average of LFP spike discharge from baseline (days −4 to −1) to the early post-KA (days 1–7) and the late post-KA phases (days 25–28), showing the evolution of epileptic spikes (n = 9 mice). Notable increase in epileptic spike frequency beyond the baseline occurred in the recorded brain areas after hippocampal KA infusion. Data are expressed as mean ± SD. (D) The average LFP spike discharge of baseline, early post-KA, and late post-KA phases in the hippocampus and cortex. n = 9 mice; one-way repeated-measures analysis of variance with Bonferroni multiple comparisons, *p < .05, **p < .01, ***p < .001. Data are expressed as mean ± SD.
RM ANOVA, $F_{2, 16} = 13.55, p < .001$; Bonferroni multiple comparisons, baseline vs. early post-KA = 1.468 ± .42 vs. 5.556 ± 2.72 spikes/min, $p = .0044$; baseline vs. late post-KA = 1.468 ± .42 vs. 6.769 ± 4.39 spikes/min, $p < .001$; $n = 9$ mice), and MCx (Figure 4D, right; one-way RM ANOVA, $F_{2, 16} = 9.605, p < .0018$; Bonferroni multiple comparisons, baseline vs. early post-KA = 1.909 ± .49 vs. 3.715 ± 1.35 spikes/min, $p = .0365$; baseline vs. late post-KA = 1.909 ± .49 vs. 4.667 ± 1.61 spikes/min, $p = .0016$; $n = 9$ mice).

The evolution as well as increased frequency of epileptic spikes within and outside the hippocampus in the early post-KA period signifies the progression of local and remote neuropathological network alteration, with increasing neuronal network excitability as the disease progresses.

### 3.5 Unilateral surgical or bilateral chemical removal of the hippocampus does not alleviate seizures in TLE mice

Our previous experiments have shown that the epileptic activities that characterize the KA-injected hippocampus are also present in cortical areas remote from the initial injection zone. Hence, the KA-injected hippocampus may not be critically essential in generating seizures in the later stage of TLE in mice. Epileptic activities are therefore likely to occur in nonhippocampal brain areas independent of the KA-injected hippocampus. Long-term follow-up studies in human TLE patients whose unilateral hippocampus has been surgically removed has shown that these patients often experience seizure activities months to years after surgery. Similarly, the removal of bilateral hippocampus in severe cases of refractory epilepsy does not confer seizure freedom on all patients who undergo bilateral hippocampectomy. These cases often demonstrate that seizures in TLE patients can arise from extrahippocampal brain regions independent of the hippocampus. We conducted two different experiments to ascertain whether seizures can arise after removing the hippocampus in TLE mice. First, we performed surgical removal of the unilateral KA-injected hippocampus in a group of mice tagged as KA-Hpctomy, and second, we performed chemical removal of the bilateral hippocampus in another cohort of mice tagged as KA-IBO, whereas a control group without hippocampus removal was tagged as KA-Only.

Mice grouped as KA-Hpctomy or KA-Only (control) were injected with KA in the dorsal hippocampus to generate a TLE mouse model, followed by 24/7 video recording. KA-Hpctomy mice were subjected to surgical removal of KA-injected hippocampus after baseline recording of spontaneous seizures, whereas the hippocampus of the KA-Only control group was not removed. The average latent period for the KA-Hpctomy and KA-Only mice was 11.4 ± 5.4 days (range = 5–21 days) and 9.2 ± 3.2 days (range = 5–17 days), respectively. Baseline spontaneous seizures in the home cage were recorded for 7 days in both groups of mice. The KA-injected hippocampus of the KA-Hpctomy mice was removed via surgical aspiration after baseline recording (day 8), and video recording was continued after surgery (Figure 5A). Histological examination showed that 81.2% ± 3.8% of the KA-injected hippocampal tissue of KA-Hpctomy mice was surgically aspirated (Figure S2). Although there was a decrease in the frequency of spontaneous seizures in the 1st, 2nd, and 3rd week after seizures began to occur again following surgical removal of the hippocampus compared to baseline frequency, this decrease was nonsignificant (Figure 5B; one-way RM ANOVA with Geisser–Greenhouse correction, $F_{1.896, 15.17} = 2.487, p = .1184$; Bonferroni multiple comparison, baseline vs. week 1 = 1.08 ± .64 vs. .76 ± .41 seizures/day, $p = .3195$; baseline vs. week 2 = 1.08 ± .64 vs. .65 ± .49 seizures/day, $p = .4976$; baseline vs. week 3 = 1.08 ± .64 vs. .51 ± .55 seizures/day, $p = .2043; n = 9$ mice). Group comparison of seizure frequency between

**FIGURE 5** Seizures continue to occur after removal of hippocampus in temporal lobe epilepsy (TLE) mice. (A) Experimental design for hippocampal kainic acid (KA) injection, and unilateral surgical or bilateral chemical removal of hippocampus in TLE mice. Mice were video recorded 24/7 after KA injection, and baseline spontaneous seizure frequency was recorded for 7 days. Mice in the KA-Hpctomy group had their KA-injected hippocampus surgically removed on day 8, whereas mice in the KA-IBO group were subsequently injected with ibotenic acid (IBO) in bilateral hippocampus on day 8. The hippocampus of the KA-Only group was neither surgically removed nor injected with IBO. Video recording continued for 3 weeks to monitor seizure frequency in mice. (B) The average seizure frequency in baseline, post-KA-Hpctomy, and post-KA-Only ($N = 9$ mice for each group; two-way repeated-measures [RM] analysis of variance [ANOVA]; NS, not significant). Data are expressed as mean ± SD. (C) The average seizure duration in baseline and post-KA-Hpctomy ($n = 9$ mice for each group; two-way RM ANOVA). Data are expressed as mean ± SD. (D) Day-to-day average seizure frequency from baseline to postbaseline for KA-Only group and to post-IBO injection for KA-IBO group. Data are expressed as mean ± SD. (E) The average seizure frequency at baseline and weeks 1–3 postbaseline in the KA-Only group and in the KA-IBO group ($n = 6$ mice for KA-IBO group, $n = 7$ mice for KA-Only group; two-way RM ANOVA). Data are expressed as mean ± SD. (F) The average seizure duration at baseline and weeks 1–3 postbaseline in the KA-Only group, and in the KA-IBO group ($n = 6$ mice for KA-IBO group, $n = 7$ mice for KA-Only group; two-way RM ANOVA). Data are expressed as mean ± SD.
the KA-Hptomy and KA-Only groups showed there is no statistical difference in baseline seizure frequency and in the weeks 1–3 after baseline seizures, suggesting that seizures in TLE mice can continue to occur in the absence of the KA-injected hippocampus (Figure 5B; two-way RM ANOVA, F3, 24 = .2751, p = .8427; Bonferroni multiple comparison, seizures/day, baseline: KA-Hptomy vs. KA-Only = 1.08 ± .64 vs. 1.29 ± 1.11 seizures/day, p > .9999; week 1: KA-Hptomy vs. KA-Only = .76 ± .41 vs. 1.32 ± .52 seizures/day, p = .8161; week 2: KA-Hptomy vs. KA-Only = .65 ± .49 vs. .77 ± .42 seizures/day, p > .9999; week 3: KA-Hptomy vs. KA-Only = .51 ± .55 vs. .70 ± 4 seizures/day, p > .9999). The surgical removal of the unilateral hippocampus of non-KA-injected naïve mice did not cause epileptic seizures, although mice were recorded for a longer period (Figure S3A–C). In addition, the duration of seizures after the removal of the unilateral hippocampus in the KA-Hptomy mice was comparable to baseline values (Figure 5C; one-way RM ANOVA with Geisser–Greenhouse correction, F2,136, 17.99 = .3166, p = .7465; Bonferroni multiple comparisons, baseline vs. week 1 = 31.11 ± 7.08 vs. 27.44 ± 9.66 s, p > .9999; baseline vs. week 2 = 31.11 ± 7.08 vs. 29.44 ± 21.18 s, p > .9999; baseline vs. week 3 = 31.11 ± 7.08 vs. 34.44 ± 21.32 s, p > .9999; n = 9 mice). Also, comparison of seizure duration between the KA-Hptomy and KA-Only mice at baseline and the weeks after baseline showed no significant difference (Figure 5C; two-way RM ANOVA, F3, 24 = .3462, p = .7922; Bonferroni multiple comparison, baseline: KA-Hptomy vs. KA-Only = 31.11 ± 7.08 vs. 22.27 ± 6.74 s, p = .7585; week 1: KA-Hptomy vs. KA-Only = 27.44 ± 9.66 vs. 22.44 ± 11.99 s, p > .9999; week 2: KA-Hptomy vs. KA-Only = 29.44 ± 21.18 vs. 19.28 ± 12.20 s, p = .5334; week 3: KA-Hptomy vs. KA-Only = 34.44 ± 21.32 vs. 20.11 ± 11.98 s, p = .1538; n = 9 mice for each group). The volume of the residual KA-injected hippocampal tissue after surgical aspiration is negligible and is insufficient to account for the severity of seizures observed after the removal of hippocampus. Because complete seizure freedom could not be achieved after removing the KA-injected hippocampus, seizure generation could have been taken over by secondary epileptic foci independent of the KA-injected hippocampus in TLE.

To exclude the possibility of seizure initiation from possible mirror focus in the contralateral hippocampus,26 we turned our attention to removing the bilateral hippocampi of TLE mice using IBO.27 Mice grouped as KA-IBO and KA-Only (control) were injected with KA in dorsal hippocampus to generate a TLE model. KA-IBO mice were further injected with IBO in bilateral hippocampi after baseline recording of spontaneous seizures, whereas the hippocampi of the KA-Only control group were not injected with IBO. The average latent period for the KA-IBO and KA-Only mice was 7.8 ± 2.2 days (range = 5–11 days) and 8.9 ± 2.6 days (range = 5–11 days), respectively. Baseline spontaneous seizures in the home cage were recorded for 7 days in both groups of mice. Bilateral hippocampi of the KA-IBO mice were lesioned through IBO injection after baseline recording (day 8), and video recording was continued for 21 days after IBO injection; similarly, KA-Only mice were also continuously video monitored after baseline (Figure 5A). Histological examination revealed that 88.5 ± 4.88% of bilateral hippocampi was removed after bilateral IBO injection of KA-IBO mice (Figure S4). A relatively stable baseline seizure frequency was recorded in the KA-IBO and KA-Only groups. Surprisingly, mice continued to have seizures in the KA-IBO group after bilateral hippocampal lesion, and seizure freedom could not be achieved after IBO lesioning of the bilateral hippocampi (Figure 5D; n = 6 mice for KA-IBO and n = 7 mice for KA-Only). Group analysis of seizure frequency revealed nonsignificant change in the frequency of seizures recorded at baseline and those recorded post-IBO in the KA-IBO group (Figure 5E; one-way RM ANOVA with Geisser–Greenhouse correction, F2,437, 12.12 = 3.372, p = .0612; Bonferroni multiple comparison, baseline vs. week 1 = 2.88 ± 3.18 vs. 1.60 ± 2.75 seizures/day, p = .0607; baseline vs. week 2 = 2.88 ± 3.18 vs. 2.05 ± 3.91 seizures/day, p = .3862; baseline vs. week 3 = 2.88 ± 3.18 vs. 1.71 ± 2.98 seizures/day, p = .1640; n = 6 mice, seizures/day). Group comparison of seizure frequency between the KA-IBO and KA-Only groups further revealed that there was no statistical difference in baseline seizure frequency or in the frequency of seizures in the weeks following hippocampal IBO lesion (Figure 5E; two-way RM ANOVA, F3, 33 = 1.131, p = .3508; Bonferroni multiple comparison, seizures/day, baseline: KA-IBO vs. KA-Only = 2.88 ± 3.18 vs. 1.94 ± 1.66 seizures/day, p > .9999; week 1: KA-IBO vs. KA-Only = 1.60 ± 2.75 vs. 2.35 ± 3.61 seizures/day, p > .9999; week 2: KA-IBO vs. KA-Only = 2.05 ± 3.91 vs. 1.10 ± 1.35 seizures/day, p > .9999; week 3: KA-IBO vs. KA-Only = 1.71 ± 2.98 vs. 2.02 ± 3.86 seizures/day, p > .9999; seizures/day; n = 6 mice for KA-IBO group and n = 7 mice for KA-Only group). It is very unlikely that IBO, being an excitotoxic agent, directly potentiates seizure activities in the epileptic mice, because only on one occasion each was a seizurelike behavior recorded from two nonepileptic naïve mice injected with IBO only (Figure S5). There was no notable difference in the duration of seizures before and after IBO lesioning in the KA-IBO group (Figure 5F; one-way RM ANOVA with Geisser–Greenhouse correction, F1,921, 9.609 = 1.131, p = .3598; Bonferroni multiple comparison, baseline vs. week 1 = 28.67 ± 14.11 vs. 19.17 ± 16.25 s, p > .9999; baseline vs. week 2 = 28.67 ± 14.11 vs. 41.33 ± 29.20 s,
data and therefore deserves further investigation. In TLE mice cannot be fully elucidated by the current studies.31-33 The activation of such remote networks in the onset of these stimulus-induced seizures. Cortical stimulation via implanted electrodes has been used to induce seizures in focal drug-resistant epilepsy.34 However, the use of a noninvasive sensory stimulus to induce seizures, as shown in our study, means our model can be adopted in epilepsy research, thereby breaking the barrier of limitations in studying epileptic seizures in TLE mice due to the stochastic occurrence of spontaneous seizures.

Some studies using mice model have attempted to abolish seizure onset and seizure frequency in TLE mice by manipulating the hippocampus.35-37 However, many of these studies generally have a mild success rate in reducing seizure frequency, and the explanation for the occurrence of seizures after silencing the hippocampus is often lacking. Identifying cortical SOZ in TLE mice in light-induced and noise-induced seizures, especially in mice subjected to light induction during the induction period, shifts our focus from concentrating solely on the hippocampus to seeing TLE as a brain-wide pathology involving remote SOZs. Although the hippocampus is dominant as the SOZ in TLE mice not exposed to any stimulus induction, whether cortical hyperexcitability plays a critical factor in increasing hippocampal excitability leading to seizure initiation in the hippocampus in these mice needs further investigation. The presence of nonhippocampal SOZs in our study could not have been because of volume conduction, because we used tungsten electrodes with impedance of 100–200 kΩ for our recording, which record electrical signals within a distance of ~200–1000 µm. Also, the volume of KA we injected, although larger than that used in other studies,10 could not have contributed to the occurrence of nonhippocampal SOZs, because the percentage occurrence of nonhippocampal SOZ in the TLE mice that were not subjected to stimulus induction in our study is similar to those reported by other studies that used a similar volume. Hence, the higher KA injection volume did not necessarily contribute to the higher percentage of extrahippocampal SOZ in TLE+Light and TLE+Noise mice. The existence of cortical SOZ in our study supports the computational model previously proposed by Słowiński et al.38 for the presence of seizure nodes outside the hippocampus in TLE.

4 | DISCUSSION

The expression of the immediate early gene, c-Fos, readily reveals neurons that have undergone an intense firing in response to various stimuli, including chemoconvulsants.28-30 Therefore, enhanced c-Fos expression in the hippocampus and cortex after spontaneous seizure suggests that the observed spatial distribution of Fos+ positive cells is due to neuronal excitation. Hippocampal and nonhippocampal neuronal networks are activated and discharged synchronously during a convulsive seizure in TLE mice, indicating the establishment of an active epileptic network that recruits cortical neurons far beyond the hippocampus. The activation of such remote networks further strengthens the possibility of epileptic activities arising outside the hippocampus, as suggested by other studies.31-33 Sensory auras such as visual and auditory auras occur in some TLE patients, and this can serve as a warning sign for an upcoming epileptic seizure.15,16 Seizure relapse after TLE surgery in these patients with preoperative auras provides supporting evidence for the existence of nonhippocampal secondary seizure focus in TLE disease. By establishing the light-induced and noise-induced seizure models in TLE mice, we have shown that exposing TLE mice to external stimuli can enhance epileptic network excitation leading to an epileptic seizure. Furthermore, that mice developed an increased frequency of light-induced or noise-induced seizures that corresponds to the induction modality used during the latent phase suggests the possibility of network remodeling during the induction period that subsequently makes TLE mice more vulnerable to seizures that can be precipitated by visual or auditory stimulation. This further reveals a critical role for the participation of cortical networks in the onset of these stimulus-induced seizures. Cortical stimulation via implanted electrodes has been used to induce seizures in focal drug-resistant epilepsy.34 However, the use of a noninvasive sensory stimulus to induce seizures, as shown in our study, means our model can be adopted in epilepsy research, thereby breaking the barrier of limitations in studying epileptic seizures in TLE mice due to the stochastic occurrence of spontaneous seizures.
Knowing whether the electrophysiological events observed in epileptic patients can be detected in the early phase of the disease and whether these changes extend beyond the hippocampus could be important in the early diagnosis of an epileptic condition, and therapeutic intervention prior to the first spontaneous seizure could be adopted early. The appearance of epileptic spikes in subsequent days after KA infusion reveals an ongoing network reorganization and increasing network excitability, showing that the disease is invading nonhippocampal neuronal networks faster than previously reported. Hence, a network wherein epileptic activities can be initiated and propagated is being formed in the early and later stages of the disease.

Seizure activities have been observed in drug-resistant TLE patients months to years after surgical resection of the epileptic hippocampus, and the probability of seizure freedom decreases with time. Although there is limited information on the causes of seizures after surgery, reasons such as the development of new epileptic foci have been proposed to explain seizure recurrence. The results from our study agree with the development of remote epileptic foci and support the existence of nonhippocampal epileptogenic networks that are either present before or evolved after the removal of the epileptic hippocampus and manifest with new seizures. Inability to stop seizure occurrence after unilateral or bilateral hippocampus removal indicates the existence of an extensive epileptic network beyond the ipsilateral and contralateral hippocampus where seizures can be initiated in the later stage of TLE. Our findings suggest that seizure initiation can occur independent of the KA-lesioned hippocampus, and removing the epileptic and contralateral hippocampus cannot stop seizure activities. Because we only conducted video monitoring and behavioral seizure analysis for TLE mice whose hippocampus injected and the contralateral hippocampus did not stop the occurrence of epileptic seizures. Therefore, the study of TLE should not exclude the participation of nonhippocampal networks, particularly cortical networks, in the progression of the disease. Our study provides new insight into the need for a comprehensive therapeutic approach that includes suppressing epileptic activities in the cortex, rather than targeting the hippocampus alone in treating TLE disease.

5 | CONCLUSIONS

This study has shown that remote nonhippocampal networks actively participate in the ongoing pathology that is associated with TLE disease. Activation of the cortex during spontaneous seizure and the occurrence of cortical and hippocampal epileptic discharges reveal that TLE is a global and not a local network disease, hence epileptic seizures can be initiated in cortical networks when TLE+Light and TLE+Noise mice are exposed to sensory stimulus (light or noise). Importantly, removing the KA-injected and the contralateral hippocampus did not stop the occurrence of epileptic seizures. Therefore, the study of TLE should not exclude the participation of nonhippocampal networks, particularly cortical networks, in the progression of the disease. Our study provides new insight into the need for a comprehensive therapeutic approach that includes suppressing epileptic activities in the cortex, rather than targeting the hippocampus alone in treating TLE disease.

AUTHOR CONTRIBUTIONS

Stephen Temitayo Bello and Jufang He designed the experiments and wrote the paper. Stephen Temitayo Bello conducted the intrahippocampal injections, in vivo experiments, immunohistochemistry, and confocal imaging. Shenghui Xu helped in the in vivo and video recording. Xiaoli and Xi Chen helped with several in vivo experiments. Junming Ren and Zhoujian Xiao worked on the autodetection program for seizures, and Xiaoxiao Wan helped in the manual checking of the seizures. Feixiu Jiang helped in immunohistochemistry experiments. Stephen Temitayo Bello, Junming Ren, and Peter Jendrichovsky plotted the figures and analyzed the data.

ACKNOWLEDGMENTS

The authors thank Mengying Chen, Ng Suet Yan, Yin Anqi, and Ng Siu Lung for administrative and technical assistance. We also thank the following charitable foundations for their generous support to J.H.: Wong Chun Hong Endowed Chair Professorship, Charlie Lee Charitable Foundation, and Fong Shu Fook Tong Foundation. This work was supported by the Hong Kong Research Grants Council, General Research Fund (11101215, 11103220, 11101818, 11102417, 11166316, 14117319, to J.H.), Collaborative Research Fund (C1014-15G, C7048-16G, to J.H.), Innovation and Technology Fund (MRP/053/18X, MRP/101/17X, GHP_075_19GD, to J.H.), Health and Medical Research Fund (3141196, 1121906, 06172456, 31571096, to J.H.), National Natural Science Foundation of China (31671102, to J.H.), and Key-Area Research and Development Program of Guangdong Province (2018B030340001, to X.L.).

CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose.

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